

CATABOLISM OF GLUCOSE DISSOLVED IN SEA WATER
BY MYIS AND POSTLARVAE OF TWO SPECIES OF
PENAEID SHRIMP¹

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ABSTRACT

Respiration rates were determined in myis and early postlarval stages in the shrimp species Penaeus aztecus and Penaeus setiferus. Rates in the same stages differed somewhat between the two species, being higher in Penaeus setiferus at the experimental temperature of 28 C and salinity of 32 ppt. On the basis of oxygen consumption per unit dry weight, rates were highest in the youngest stages and lower in the older stages. The ability of these organisms to catabolize D-glucose dissolved in seawater was studied, using uniformly labeled ¹⁴C-D-glucose. Respiratory CO₂ was collected, and the radioactivity determined by liquid scintillation counting. Catabolism of the dissolved D-glucose on a per unit dry weight basis was not different between the two species at the same stage of development and was highest in the earlier stages, decreasing with increasing animal size. The results indicate that dissolved D-glucose contributes to the total catabolic requirements in these animals. The contribution to total respiration made by dissolved glucose was very small. The potential for this contribution needs further study.

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INTRODUCTION

The occurrence of dissolved organic compounds, including carbohydrates, in seawater in considerable amounts has been widely reported. The composition of dissolved organic compounds in seawater was discussed in a review by Wagner (1969) and in particular, the occurrence and role of glucose in seawater was investigated by Vacarro et al. (1968). Other recent studies include those by Biggs and Wetzel (1968), Khailov and Burlakova (1969), Bohling (1970), and Thomas (1971).

Since Putter (1909) speculated on the possibility that these dissolved organics might contribute significantly to the nutrition of marine animals, many investigators have reported uptake and utilization of dissolved amino acids, glucose, and other small organic molecules from seawater by estuarine and marine invertebrates. The literature has been reviewed by Stephens (1972). In virtually all of these investigations, adults have been used rather than larvae or juveniles. Also, in spite of the commercial importance of the penaeid shrimp, the possibility of utilizing dissolved organics as a source of nutrition for shrimp has been virtually ignored. In particular, no attempts have been made to measure respiration and collect respired radioactive CO₂ concurrently for counting so as to provide for a direct estimate of the contribution of dissolved organic matter to respiratory catabolism.

The purpose of this investigation was to determine if late larval and early postlarval stages of two commercially important shrimp species, Penaeus aztecus and Penaeus setiferus, could catabolize significant amounts of D-glucose dissolved in seawater. The basis for this work was the demonstration by Lawrence (1972) that these animals could absorb and metabolize dissolved D-glucose from seawater.

The data presented here have significance from three aspects: (1) the respiration rates add to the body of information already available on respiration rates in various marine animals, particularly in the crustaceans and more particularly in immature forms, and thus make a contribution to comparative physiology; (2) they provide information that can be used to help in estimating the nutritional requirements of shrimp in a mariculture situation; and (3) they give an indication of the potential for the use of dissolved organic nutrients to meet part of the total metabolic requirements.

MATERIALS AND METHODS

Mysis and early postlarvae (up to 9-day-old postlarva) of the brown shrimp P. aztecus and the white shrimp P. setiferus were obtained from Dow Chemical Company at Freeport, Texas; Ralston Purina Company at Crystal River, Florida; and the National Marine Fisheries Service at Galveston, Texas. After acclimation to room temperature and salinity of 32 ppt, several samples of animals

were isolated, counted, weighed, dried, and then reweighed. A known number of animals were washed and introduced into flasks of the Gilson differential respirometer, in 3.5 ml of medium consisting of artificial seawater at 32 ppt salinity, with oleandomycin and oxytetracycline at 50 and 125, 100 and 250, or 200 and 500 µg/ml, respectively. An oleandomycin and oxytetracycline concentration of 50 µg/ml and 125 µg/ml is bacteriostatic whereas the higher concentrations are better than 99% bactericidal (Chan and Lawrence, 1974). The animals were preincubated in this medium for 6 hours at 25 C and 80 oscillations per minute. Respiration rates reported in this paper were taken during the sixth hour of preincubation. At the end of this time, either uniformly labeled ¹⁴C-D-glucose or uniformly labeled ¹⁴C-D-mannitol was added to each flask, in 0.5 ml of the same medium as that used for preincubation, to give a final volume of 4.0 ml and sugar concentration of 10⁻⁵ M. Specific activity of the labeled D-glucose, after dilution with cold D-glucose, was 5.4 microcuries/micromole. At a concentration of 10⁻⁵ M, and 4.0 ml solution, each flask contained 0.20 microcuries of radioactivity. Specific activity of the D-mannitol after dilution was 3.5 microcuries/micromole, or 0.14 microcuries per flask. The animals were incubated for 8 hours in this medium at the same temperature and frequency of shaking as used in the preincubation period. Respiration was measured during the second, fourth, sixth, and eighth hours of this period. Respired CO₂ was trapped on circles of filter paper pleated and placed into the center well of the flask with 0.1 ml of 20% KOH. At the end of the incubation period, 0.5 ml of 0.3 N HCl was added from the side arm of the flask to drive off dissolved CO₂ from the medium. After continued shaking for 1 hour, the filter paper was removed from the center well and placed into a liquid scintillation cocktail for counting. The animals were removed from the medium and counted. The number of animals was multiplied by the previously determined dry weight per animal and the result used to compute respiration rates. The count of radioactivity in the filter paper wicks was used to calculate the amount of dissolved sugar catabolized and the percent contribution that the dissolved sugar had made to the total respiration during the incubation period. The percent recovery of radioactivity was 89.1 ± 1.6 SEM.

RESULTS

The weight-specific respiration rates and per-animal respiration rates for the sixth hour of preincubation are reported in Table 1 for P. setiferus and in Table 2 for P. aztecus. Respiration rates fluctuated considerably during the first 5 hours but usually stabilized by the sixth hour. Over the remainder of the incubation period, the respiration rates tended to decline somewhat, either because of the antibiotics or from lack of food, or both. A slight, though consistent, stimulatory effect of D-glucose on respiration was seen during the incubation period, when compared with D-mannitol. The experimental design permitted not only a preincubation period in antibiotics to produce maximum reduction of bacterial populations before adding the sugars, but also the stabilization of respiration rates at a time fairly early

the influence of body weight is cancelled out.

Of more practical importance in a mariculture situation is that if the log-log plot of the respiration rate versus the body weight is linear throughout development, and values for a and k are known, the respiration rate for the different stages of development can be estimated by simply weighing the animals. This procedure will permit accurate estimation of minimum nutritional requirements for the different stages of development.

Contribution of Dissolved D-Glucose to Respiration

Although numerous investigators have shown that marine and estuarine invertebrates can remove dissolved organics from seawater, Stephens and Schinske (1961) examined several arthropods and found that among 35 genera and 11 phyla tested, only the arthropods failed to remove amino acids from solution. However, McWhinnie and Johanneck (1966) found that Antarctic euphausiids were able to take up acetate and glucose from seawater. In an attempt to clarify the situation, Anderson and Stephens (1969) showed that apparent uptake of amino acids from seawater by various small marine crustaceans is very slow, if it occurs at all. They suggested that epiflora were responsible for most of the apparent uptake by arthropods found by other investigators. However, most of their work was with adults. As was mentioned earlier, Lawrence (1972) has demonstrated the ability of larval and early postlarval shrimp to absorb dissolved D-glucose against an apparent concentration gradient. The production of $^{14}\text{CO}_2$ in the present study shows that the D-glucose is not only absorbed but can enter catabolic pathways and contribute to the energy needs of these animals.

The conclusion of Anderson and Stephens (1969) that epiflora can be responsible for most of the measured uptake of dissolved organics was borne out in studies preliminary to this one in which no antibiotics were used. The amount of $^{14}\text{CO}_2$ derived from D-glucose was much greater than when antibiotics were used. In this study, efforts were made to eliminate most of the epiflora and to evaluate the effects of those which could not be eliminated, by comparing the rate of catabolism of ^{14}C -D-mannitol to that of ^{14}C -D-glucose.

Since D-mannitol is not known to be catabolized by animal tissues, the catabolism of D-mannitol in these experiments was taken to be an estimate of the activity of bacteria and other microorganisms associated with the shrimp. It is apparent from Tables 3 and 4 that even when the antibiotic types and concentrations used were sufficient to reduce the bacterial populations by more than 99% (Chan and Lawrence, 1974) catabolism of the sugars was not completely eliminated. Although the higher concentrations of oleandomycin and oxytetracycline usually reduced the catabolism of D-mannitol more than did the lower concentrations used, equal concentrations of antibiotics were not always equally effective (compare the results for 6-day postlarvae and 7-day postlarvae in Table 4). This variability is probably due

to a different flora (qualitatively and/or quantitatively) known to be present from different hatches of shrimp from the same hatchery or from different hatcheries.

The percent of total respiration supported by the dissolved D-glucose was very small in these experiments. It should be noted that the incubation period was relatively short. The specific activity of the free glucose pool in the animal tissues would be expected to be quite low due to the large dilution effect upon the accumulated ^{14}C -D-glucose by the D-glucose present in the free monosaccharide pool within the animal. Bergreen et al. (1961) reported that when uniformly labeled ^{14}C -glucose was injected into the ventral sinus of the shore crab *Hemigrapsus nudus*, the amount of $^{14}\text{CO}_2$ collected was greatly reduced if the blood glucose level was elevated by injection of unlabeled glucose. It should also be noted that the percent of ^{14}C which they recovered in CO_2 ranged from as little as 1.6% in 12 hours to a maximum of 19.2% in 24 hours, varying with sex and stage of the molt cycle. Other estimates of respiration of absorbed nutrients run between 30 and 50% for the bivalve *Mallemus* (Sorokin and Wyshkwarzen, 1973) and about 50% for *Nereis* (Southward and Southward, 1972).

Because of the necessity in these experiments for terminating incubation by acidification to release labeled CO_2 from solution and from carbonates in the exoskeleton, it was not possible to measure total uptake of the accumulated labeled monosaccharide into the animals. The loss of radioactivity from the experimental system ($89.1\% \pm 1.6$ SEM recovery) probably is due to accumulated ^{14}C into the shrimp in the form of D-glucose or metabolites of D-glucose.

Although the ability of mysis and early postlarval penaeid shrimp to catabolize dissolved glucose has been shown in these experiments, more information will be required before an accurate estimate can be made of the potential contribution that it can make to total metabolic requirements.

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